Engineers (2005); Francis P. Garvan-John M. Olin Medal, American Chemical Society (2005); Carothers Award of the American Chemical Society Delaware Division (2003); and David Perlman Lecture Award, American Chemical Society, Biochemical Technology (2003).

5. I am an author or co-author of numerous publications, including several research publications in peer reviewed scientific journals. I am also a named inventor or co-inventor on several patents and patent applications.

6. Details of my education, professional experience and publications are include in my *Curriculum Vitae*, which is submitted as Exhibit 1.

I. BACKGROUND

7. I have read and am familiar with U.S. Patent No. 6,867,031 entitled "AMYLASE VARIANTS" ("the '031 patent"). I understand, on information and belief, that this patent issued from an application that was first filed in 1995.

8. I also understand, on information and belief, that the '031 patent is the subject of a law-suit between Novozymes A/S ("Novozymes"), and the parties Genencor International, Inc. ("Genencor") and Enzyme Development Corporation ("EDC"). In particular, I understand that Genencor and EDC have been accused of making and selling a product in the United States, called Spezyme Ethyl, that infringes the '031 patent.

A. *Alpha-Amylase Enzymes*

9. The '031 patent is concerned with variants of a certain kind of enzyme, called an alpha-amylase. The variant alpha-amylase enzymes that are the subject of the '031 patent have properties that make them useful for certain industrial applications.

10. Enzymes, including alpha-amylase enzymes, are proteins that function to facilitate biochemical reactions. Alpha-amylases are one specific type of enzyme. Alpha-

amylases catalyze a chemical reaction to degrade starches into smaller polymers of glucose. Specifically, alpha-amylases facilitate a chemical reaction that breaks particular chemical bonds, called the alpha-1,4-glucosidic bonds, in starch. Because a molecule of water is consumed during this chemical reaction, it is known as the "hydrolysis" of the alpha-1,4-glucosidic bond. Alpha-amylase enzymes are therefore said to "hydrolyze" alpha-1,4-glucosidic bonds in starch.

11. Because the alpha-1,4-glucosidic bonds degraded by alpha-amylases are internal bonds within a starch molecule, these enzymes are sometimes called "endoamylases." The "endo-" prefix indicates that they degrade internal bonds in starch.

12. Alpha-amylases are made naturally by many different organisms. For example, different species of bacteria and fungi also make and use alpha-amylases to degrade starch. The hydrolysis of starch into smaller polymers is also an important chemical reaction in many industries. Alpha-amylase enzymes can be used in those industries to facilitate that chemical reaction. For example, according to the '031 patent, alpha-amylase enzymes can be used industrially to liquefy starch, to desize textiles, to modify starch for use in paper, to digest starches in laundry and dishwashing detergents, and to create sugars during brewing and baking. *See* the '031 patent at 1:35-41.[1] *See also*, *Id.* at 19:53-21:49.

13. The alpha-amylases produced by different organisms are similar in that they all assist in the same chemical reaction: the hydrolysis of alpha-1,4-glucosidic bonds in starch. However, alpha-amylases produced by different types of organisms (*e.g.*, by different species of

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[1] The convention used in this Declaration, when referring to a U.S. patent, is to cite column and line number(s) separated by a colon. In this case, when referring to the '031 patent, 1:35-41 refers to column 1, lines 35-41.

bacteria and fungi) will often differ from one another in some of their properties. For example, individual alpha-amylases may differ in properties such as their "thermostability" (*i.e.*, their ability to catalyze chemical reactions at higher temperatures), their "pH optimum" (*i.e.*, their ability to catalyze chemical reactions under different pH conditions), their susceptibility to damage by oxidation, or their stability in the presence of certain minerals or metals. Because of these differences, certain alpha-amylases may be preferred over others for certain industrial applications.

B. Proteins and Amino Acid Sequences

14. Enzymes, including alpha-amylases, are proteins. All proteins, including alpha-amylases and other enzymes, are linear chains of smaller molecules, called "amino acids," that are joined together by chemical bonds. More specifically, each amino acid in a protein is joined to its neighboring amino acids in the chain by a type of carbon-nitrogen chemical bond called a "peptide bond." The resulting chain of amino acids therefore has one end with an amino chemical group, which is called the "N-terminus", and a carboxyl chemical group on the other end, which is called the "C-terminus".

15. There are twenty (20) different amino acids that occur in nature, and each protein chain includes a series of these twenty amino acids joined together, by peptide bonds, in a particular order. The order of amino acids in a protein is known as its "amino acid sequence." The amino acid sequence of a particular protein is generally reported by listing the identities of each amino acid residue, from the N-terminus to the C-terminus, in the order that they appear in the protein chain.

16. Typical protein sequences are actually illustrated in the '031 patent, for example, at Figure 1. The amino acid sequences in Figure 1 of the '031 patent are denoted using a

standard, scientific nomenclature that assigns a one letter abbreviation to each one of the twenty different amino acids found in nature. For example, one amino acid, known as threonine, is abbreviated as "T". Another amino acid, known as glycine, is abbreviated as "G".

17. For example, the first five amino acid residues in Figure 1, line 1 of the '031 patent are denoted "HHNGT". This denotes that the first amino acid residue in that protein is a histidine ("H"), followed, in order, by another histidine ("H"), asparagine ("N"), glycine ("G") and threonine ("T").

18. A particular amino acid residue within a sequence can be denoted by giving the single-letter abbreviation for the name of that amino acid, followed by a number indicating its position in the amino acid sequence. For example, the fifth amino acid residue of the sequence at Figure 1, line 1 of the '031 patent, which is threonine ("T"), can be denoted as T5.

19. A protein's individual characteristics are determined by the number of amino acids in the protein chain, by the specific identities of the amino acids in the protein chain and, in particular, by the sequence of those amino acids in the protein chain. These characteristics include the protein's function (for example, the particular chemical reaction that an enzyme protein catalyzes), as well as the conditions under which it performs that function. Different proteins that have the same or similar functions will typically have similar, although not identical, amino acid sequences.

20. For example, alpha-amylases produced by different organisms are similar in that they all assist in the same chemical reaction: the hydrolysis of alpha-1,4-glucosidic bonds in starch. Consequently, alpha-amylases produced by different organisms also have similar amino acid sequences. However, the amino acid sequences of different alpha-amylases are not identical. Differences in the amino acid sequences of different alpha-amylases can give rise to

different properties, such as thermostability, pH optimum, susceptibility to oxidation, and/or stability in the presence of certain minerals or metals.

C. *Alignment and Comparison of Amino Acid Sequences*

21. Because the properties of a particular protein are determined by its amino acid sequence, it can be informative to compare the amino acid sequences of different proteins. This can be done by "aligning" the amino acid sequences with one another to achieve the best juxtaposition of amino acids that are common to both proteins. If the two sequences are very similar, then an alignment can often be performed by eye. Usually, however, alignments of amino acid sequences are made using known computer programs. One such program, the GAP computer program, is mentioned in the '031 patent at 4:36-45.

22. Figure 1 of the '031 patent illustrates an exemplary alignment of amino acid sequences. According to the '031 patent, these are the amino acid sequences of SEQ ID NOS:1-3 and 7. *See* the '031 patent at 29:38-45. The '031 patent also includes a Sequence Listing, with amino acid sequences identified as SEQ ID NOS:1-3 and 7. The patent states that these are the amino acid sequences of alpha-amylases obtained from Bacillus strains NCIB 12512 (SEQ ID NO:1 and NCIB 12513 (SEQ ID NO:2), from *Bacillus stearothermophilus* (SEQ ID NO:3), and from *Bacillus* sp #707 (SEQ ID NO:7). *Id.* at 7:24-40.[2]

23. As explained above, amino acid sequences are aligned with each other to achieve the best possible juxtaposition of individual or groups of amino acids that are common to both

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[2] I have noticed that there are some differences between the amino acid sequence identified as sequence 3 in the '031 patent's Figure 1, and the amino acid sequence at SEQ ID NO:3 of the patent's Sequence Listing. However, these differences do not affect my analysis of conclusions in this Declaration.

sequences. To achieve the best possible juxtaposition, it is sometimes necessary to introduce one or more "gaps" into an amino acid sequence to optimize its alignment with one or more other sequences. This can be seen in Figure 1 of the '031 patent. For the sequence numbered 3 in that figure, the alignment results in a gap at the position aligning with amino acid number 175 of the sequence at line 1. This gap is indicated in Figure 1 by a dash ("-") written in amino acid sequence 3 of that Figure. *See*, the '031 patent at 29:48-54.

24. This can be described by saying that the amino acid sequence at line 3 of Figure 1 is "missing" or "lacking" the amino acid equivalent to position 175 of the sequence at line 1; or as simply "missing" or lacking amino acid N175 of that sequence.[3]

25. The amino acids of two or more aligned sequences can also be compared, to see if they are alike or different. For example, referring again to the alignment shown in '031 patent Figure 1, the amino acid at position 19 in line 1 is an asparagine (N19). This amino acid aligns with an aspartic acid (D) in the sequence at line 3 of Figure 1. Hence, sequence 3 in figure is said to have an aspartic acid (D) at the position "equivalent to" amino acid 19 of the sequence at line 1.

26. The '031 patent uses this nomenclature. For example, the patent states (at 9:19-27) that:

> the term "equivalent position" denotes a position which, on the basis of an alignment of the amino acid sequence of the parent [alpha]-amylase in question with the "reference" alpha-amylase amino acid sequence in question (for example, the sequence shown in SEQ ID No. 1) so as to achieve juxtapositioning of amino acid residues/regions which are common to both, corresponds most closely to (*e.g.* is occupied by the same amino acid residue as) a particular position in the reference sequence in question.

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[3] Note that this amino acid is written here as "N175", because the amino acid at position 175 in that sequence is an asparagine ("N").

27. It is also possible to assess how similar two aligned amino acid sequences are to each other. Typically, this is done by calculating the percentage of amino acids in the aligned sequences that are identical. The '031 patent uses this definition. In particular, the patent states that:

> [a]n amino acid sequence is considered to be X% homologous to the parent [alpha]-amylase if a comparison of the respective amino acid sequences, performed via known algorithms ... reveals an identity of X%

See, the '031 patent at 4:36-40. This passage uses the term "percent homologous", and defines that term to mean the same thing as "percent identity."

D. Proteins Are Encoded By DNA

28. The proteins produced by a living organism are determined by that organism's genes. Each protein is encoded by a gene that specifies that particular protein's amino acid sequence. With the knowledge of the genetic code, one can ascertain the amino acid sequence of a protein by determining the coding DNA sequence of the gene for that protein.

29. The protein encoded by a particular DNA sequence can have its amino acid sequence modified from that encoded by the DNA. For example, proteins can be modified by processes that are generally referred to as "post-translational modification."

30. One common example of post-translational modification occurs during the secretion of proteins by cells. Typically, DNA that encodes a protein for secretion will also encode a short sequence of amino acids called a "secretion signal" or a "signal peptide." The secretion signal typically comprises a sequence of approximately 20-30 amino acids at the N-terminus of the protein. When this DNA is expressed in a cell, the cell will initially produce a protein having the secretion signal at its N-terminus. The cell will then secrete the protein into the medium outside of the cell. In this process, the secretion signal is cleaved from the protein.

Hence, the "mature" protein that is ultimately secreted by the cell will be about 20-30 amino acids shorter on its N-terminus than is the "precursor" protein (*i.e.*, the protein with the secretion signal) actually encoded by the DNA.

31. Alpha-amylase enzymes are one example of secreted proteins. DNA encoding an alpha-amylase encodes a secretion signal in addition to the alpha-amylase sequence. The secretion signal is then cleaved when the protein is secreted by the cell, thereby giving rise to the "mature" alpha-amylase.

32. Other examples of how a protein sequence can be modified are also known. For example, many cells contains enzymes called "proteases" that may degrade certain proteins at either the N-terminus or C-terminus. As a result, the "mature" protein that is actually expressed may be truncated, in that it has fewer amino acids at either the amino or carboxy-terminus than are actually encoded by the protein's coding DNA.

E. Genetic Engineering Technology

33. DNA can also be manipulated through the technology of genetic engineering. For example, organisms can be genetically engineered to produce new or modified proteins.

34. Using genetic engineering technology, a gene can be isolated from a cell or organism, and transferred to the same or a different cell or organism in which the DNA can replicate and drive production of the protein it encodes. The recipient cell or organism is termed a "host." In this way the new (or "transformed") host can be made to produce the protein encoded by the DNA introduced into that host. Enzymes and other proteins (including alpha-amylases) that are used in industry are typically made in this way.

35. The coding DNA for a protein can be modified so that it encodes a version of the protein with one or more changes in its amino acid sequence. Typically, the protein encoded by

a DNA sequence that is modified in this way is referred to as a "variant" protein, whereas the protein encoded by the original coding DNA is called the "parent" protein. The amino acid sequence of a parent can be changed by replacing or "substituting" one or more amino acids residues in the parent sequence with other amino acid residues, by removing or "deleting" one or more amino acid residues from the parent, by adding or "inserting" one or more residues into the parent sequence, or by a combination of the above.

36. The '031 patent uses also uses this nomenclature. For example, the '031 patent describes modifying DNA sequences that encode a parent alpha-amylase, to produce variant alpha-amylase proteins. In particular, the patent states (at 12:49-52) that:

> [t]he DNA sequence encoding a parent [alpha]-amylase may be isolated from any cell or microorganism producing the alpha-amylase in question, using various methods well known in the art.

The '031 patent then goes on to explain (*Id.* at 13:23-25) that:

> [o]nce an [alpha]-amylase encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced ....

II. THE '031 PATENT CLAIMS

37. I have read and considered the claims of the '031 patent. It is my understanding that the terms of the claims have their ordinary meaning in the context and field of this patent's invention, unless it appears from the patent and its file history that the words were used differently by the inventors.

A. *Claim 1 of the '031 Patent*

38. Claim 1 of the '031 patent reads as follows:

> A variant of a parent *Bacillus stearothermophilus* alpha-amylase, wherein the variant has an amino acid sequence which has at least 95% homology to the parent *Bacillus stearothermophilus* alpha-amylase and comprises a deletion of

amino acids 179 and 180, using SEQ ID NO:3 for numbering, and wherein the variant has alpha-amylase activity.

39. The preamble of claim 1 refers to "[a] variant of a parent *Bacillus stearothermophilus* alpha-amylase." As explained above, an "alpha-amylase" is a particular type of enzyme that catalyzes the breakdown, or "hydrolysis," of certain chemical bonds (the alpha-1,4-glucosidic bonds) in starch.

40. *Bacillus stearothermophilus* is a species of bacteria that naturally produces an alpha-amylase enzyme.[4] Hence, a "*Bacillus stearothermophilus* alpha-amylase" is an alpha-amylase encoded by a gene from that species of bacteria.

41. The preamble in claim 1 refers to both a "parent" *Bacillus stearothermophilus* alpha-amylase and a "variant" thereof. These terms, "variant" and "parent" have generally accepted meanings in the field of molecular biology and biotechnology. In particular, and as I have explained above, a "variant" protein is a protein with one or more changes in its amino acid sequence (*i.e.*, with one or more amino acid substitutions, insertions or deletions) when compared to an unmodified or "parent" protein.

42. Hence, I find that claim 1 is directed to a "variant" alpha-amylase enzyme (*i.e.*, an enzyme that breaks down alpha-1,4-glucosidic bonds in starch) that can be derived from a "parent" alpha-amylase of the bacterial species *Bacillus* (*Geobacillus*) *stearothermophilus*. However, the variant alpha-amylase has a different amino acid sequence (*i.e.*, with one or more amino acid substitutions, insertions, or deletions) when compared to the "parent" alpha-amylase from *Bacillus* (*Geobacillus*) *stearothermophilus*.

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[4] The accepted name for this species of bacteria (*i.e.*, for *Bacillus stearothermophilus*) has changed since 1995, when the application leading to the '031 patent was first filed. *Geobacillus stearothermophilus* is now an accepted name for this species of bacteria.

43. Claim 1 also specifies that "the variant has at least 95% homology to the parent *Bacillus stearothermophilus* alpha-amylase". As explained above, it is routine to compare amino acid sequences of different proteins by "aligning" the different sequences to achieve the best juxtaposition of amino acids that are common to both proteins, and then determining the percentage of amino acids that are identical in the aligned sequences.

44. The '031 patent describes comparing amino acid sequences in this way. In particular, the '031 patent explains (at 4:36-40) that:

> [a]n amino acid sequence is considered to be X% homologous to the parent [alpha]-amylase if a comparison of the respective amino acid sequences, performed via known algorithms, such as the one described by Lipman and Pearson in *Science* 227 (1985) p. 1435, reveals an identity of X%. The GAP computer program from the GCG package, version 7.3 (June 1993), may suitably be used, employing default values for GAP penalties....

It is clear from this passage that the '031 patent is using the terms "percent homology" and "percent identity" interchangeably.

45. From the foregoing, I find that claim 1 requires the amino acid sequence of the variant, when aligned and compared to the sequence of the parent alpha-amylase (*e.g.*, using the GAP computer program) is revealed to have a percent identity of 95% or more to the parent alpha-amylase.

46. Claim 1 additionally states that the variant "comprises a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering". The deletion of an amino acid residue in a variant protein refers to the removal of that amino acid residue, through mutation or modification of coding DNA for a parent protein.

47. From the foregoing, I find that claim 1 requires the variant alpha-amylase sequence, when aligned with SEQ ID NO:3 (*e.g.*, using the GAP computer program), to have

"gaps" or deletions at the positions aligning with amino acid residues 179 and 180 in SEQ ID NO:3.

48. I have been told that the word "comprises", when used in patent claims, has an open ended meaning that does not exclude additional, unrecited components. Claim 1 states that the variant "*comprises* a deletion of amino acids 179 and 180" (emphasis added). I therefore find that the language of claim 1 does not exclude the possibility of additional, unrecited components in the variant's amino acid sequence; including the possibility of other, unrecited amino acid substitutions, insertions or deletions.

49. Finally, claim 1 states that "the variant has alpha-amylase activity". I have already explained that alpha-amylase enzymes are characterized as having a particular catalytic activity. In particular, alpha-amylases catalyze the hydrolysis of alpha-1,4-glucosidic bonds in starch.

50. From the foregoing, I find that claim 1 of the '031 patent claims a "variant" with a different amino acid sequence (*i.e.*, having one or more substitutions, insertions or deletions) from a "parent" alpha-amylase (*i.e.*, an enzyme that breaks down chemical bonds in starch) of the bacteria species *Bacillus* (*Geobacillus*) *stearothermophilus*. When aligned with the parent alpha-amylase's amino acid sequence, the variant's amino acid sequence is more than 95% identical to the parent's. In addition, the variant's amino acid sequence, when aligned with SEQ ID NO:3 of the '031 patent, contains a deletion at positions aligning with residues 179 and 180 of SEQ ID NO:3 (and may also contain other amino acid deletions, substitutions or additions). The claimed variant also has alpha-amylase activity – *i.e.*, it can break down or "hydrolyze" certain chemical bonds (the alpha-1,4-glucosidic bonds) in starch.

B. ***Claim 3 of the '031 Patent***

51. Claim 3 of the '031 patent reads as follows:

> A variant alpha-amylase, wherein the variant has at least 95% homology to SEQ ID NO:3 and comprises a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering and wherein the variant has alpha-amylase activity.

52. The preamble of claim 3 recites "[a] variant alpha-amylase". I have already discussed the meaning of these terms – "variant" and "alpha-amylase" – with respect to claim 1. I find that they are used the same way and, hence, have the same meaning for claim 3.

53. Claim 3 also specifies that "the variant has at least 95% homology to SEQ ID NO:3". I have already explained, with respect to claim 1, that the '031 patent uses the terms "percent homology" and "percent identity" interchangeably. I therefore find that claim 3 requires the amino acid sequence of the variant, when aligned and compared to SEQ ID NO:3 of the '031 patent (*e.g.*, using the GAP computer program), is revealed to have a percent identity of 95% or more to SEQ ID NO:3.

54. Claim 3 additionally states that the variant "comprises a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering". I have already discussed this limitation with respect to claim 1. I find that it is used the same way and, hence, has the same meaning in claim 3.

55. As stated above, I understand that the word "comprises", when used in patent claims, has an open ended meaning that does not exclude additional, unrecited components. Claim 3, like claim 1, states that the variant "*comprises* a deletion of amino acids 179 and 180" (emphasis added). I therefore find that the language of claim 3 does not exclude the possibility of additional, unrecited components in the variant's amino acid sequence; including the possibility of other, unrecited amino acid substitutions, insertions or deletions.

56. Finally, claim 3 states that "the variant has alpha-amylase activity." This limitation is also recited in claim 1. I have already explained that it means the variant enzyme has the catalytic activity of an alpha-amylase – *i.e.*, it catalyzes the hydrolysis of alpha-1,4-glucosidic bonds in starch. I find that this limitation is used the same way, and therefore has the same meaning in claim 3.

57. From the foregoing, I find that claim 3 of the '031 patent claims a "variant" with a different amino acid sequence (*i.e.*, having one or more substitutions, insertions or deletions) from a "parent" alpha-amylase enzyme (*i.e.*, an enzyme that breaks down chemical bonds in starch). When aligned with the '031 patent's SEQ ID NO:3 the amino acid sequence of the variant protein is more than 95% identical to SEQ ID NO:3. However, the variant alpha-amylase amino acid sequence, when aligned with SEQ ID NO:3 of the '031 patent, contains a deletion at positions aligning with residues 179 and 180 of SEQ ID NO:3 (and may also contain other amino acid deletions, substitutions or additions). The claimed variant also has alpha-amylase activity – *i.e.*, it can break down or "hydrolyze" certain chemical bonds (the alpha-1,4-glucosidic bonds) in starch.

III. THE SPEZYME ETHYL PRODUCT

58. I have been told that Genencor makes and sells, through its distributor EDC, an alpha-amylase product called Spezyme Ethyl. I have reviewed documents pertaining to the Spezyme Ethyl product, which are discussed in detail below. From that review, it is my opinion and belief that Genencor's Spezyme Ethyl product contains a variant of a parent *Bacillus stearothermophilus* alpha-amylase that satisfies each and every one of the limitations in '031 patent claims 1 and 3.

A. ***Spezyme Ethyl Contains a Variant of a B. stearothermphilus Alpha-Amylase***

59. I have been given a document entitled "SPEZYME® ETHYL: High Performance Alpha-Amylase for Fuel Ethanol Processes" (Exh. 2), that I am told was published by Genencor. I have reviewed this document and find that it describes Genencor and EDC's Spezyme Ethyl product. In particular, this document states that the "SPEZYME® ETHYL enzyme contains a thermostable starch hydrolyzing [alpha]-amylase … that is derived from a genetically modified strain of *Geobacillus stearothermophilus*."

60. *Geobacillus stearothermophilus* is a presently accepted name of the bacteria species that was commonly known as *Bacillus stearothermophilus* in 1995, when the applications leading to the '031 patent were first filed. Hence, Exhibit 2 tells me that Genencor's Spezyme Ethyl enzyme contains an alpha-amylase derived from a genetically modified strain of *Bacillus stearothermophilus*.

61. I have been given and have reviewed a document labeled "Spezyme Ethyl Amino Acid Sequence," a copy of which is provided here at Exhibit 3. This document shows an amino acid sequence, which I understand to be the amino acid sequence determined by Novozymes for the protein contained in the Spezyme Ethyl product.

62. I have also been given, and have also reviewed, a document labeled "ATCC 31,195 Alpha-Amylase Amino Acid Sequence". A copy of that document, which also contains an amino acid sequence, is attached to this Declaration at Exhibit 4. I understand that the amino acid sequence in this document is the sequence determined by Novozymes for a mature alpha-amylase encoded by the alpha-amylase gene from a natural isolate of *Bacillus*

(*Geobacillus*) *stearothermophilus*, called the "ATCC 31,195 strain".[5] Hence, the ATCC 31,195 alpha-amylase is one example of a "parent *Bacillus* [*Geobacillus*] *stearothermophilus* alpha-amylase".

63. I have been given two additional documents, entitled "GAP Alignment: ATCC 31,195 Alpha-Amylase to Spezyme Ethyl (Old Matrix)" and "GAP ALIGNMENT: ATCC 31,195 Alpha-Amylase to Spezyme Ethyl (New Matrix)". Copies of these documents are attached to this Declaration at Exhibits 5 and 6, respectively. I have reviewed both these documents, and find that they show an alignment of the Spezyme Ethyl alpha-amylase at Exhibit 3 to the parent ATCC 31,195 alpha-amylase sequence at Exhibit 4.

64. I understand, on information and belief, that the sequence alignment at Exh. 5 was made with a current version of the GAP computer program (from version 10 of the GCG package) but using what were the default parameters for version 7.3 of that program (the version mentioned in the '031 patent at 4:36-45). I therefore understand that the alignment and the other output generated are identical to what a person would have obtained by using version 7.3 of the GAP program with default parameters to align these two sequences.

65. I also understand, on information and belief, that the sequence alignment at Exh. 6 was made with a current version of the GAP program (from version 10 of the GCG package) using the default parameters for that version. However, when I compare the sequence alignments at Exhibits 5 and 6 I find that they are identical.

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[5] The ATCC 31,195 strain is a strain of *Bacillus* (*Geobacillus*) *stearothermophilus* that has been deposited with, and can be obtained from the American Type Culture Collection or "ATCC". The ATCC is a repository of microorganisms and other biological material. ATCC 31,195 refers to the "accession number" that the ATCC uses to identify this particular microorganism deposit.

66. I find, from the alignment in these exhibits, that the Spezyme Ethyl alpha-amylase differs from the parent ATCC 31,195 alpha-amylase by having a deletion of residues R179 and G180 in the parent ATCC 31,195 alpha-amylase sequence. Also, there are three amino acid residues at the C-terminus of the parent ATCC 31,195 alpha-amylase sequence (residues 487-489) that are not present in the Spezyme Ethyl sequence. Apart from these differences, the two amino acid sequences are identical.

67. Hence, the Spezyme Ethyl alpha-amylase can be obtained by deleting amino acids R179 and G180 of an original "parent" alpha-amylase -- namely, the alpha-amylase from *Bacillus* (*Geobacillus*) *stearothermophilus* strain ATCC 31,195.[6] I therefore conclude that the Spezyme Ethyl protein is a variant of a parent *Bacillus stearothermophilus* alpha-amylase.

B. *The Spezyme Ethyl Alpha-Amylase Has More Than 95% Homology to a Parent* Bacillus stearothermophilus *Alpha-Amylase*

68. As explained above, Exhibits 5 and 6 both show identical alignments of the Spezyme Ethyl alpha-amylase (Exh. 3) to the ATCC 31,195 alpha-amylase (Exh. 4). I understand that these alignments were made using the GAP computer program mentioned in the '031 patent.

69. The sequence alignments at Exhibits 5 and 6 also show the "percent identity" that the GAP computer program calculated for those alignments. In both cases, the percent identity is 100%, which is greater than 95%.

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[6] The Spezyme Ethyl alpha-amylase can also be obtained by changing only a few amino acids in the sequence of other parent alpha-amylases from *Bacillus stearothermophilus*. For example, the '031 patent states that the amino acid sequences of SEQ ID NO:3 and of Sequence 3 in Figure 1 of that patent are also examples of parent alpha-amylases from *Bacillus stearothermophilus*. As explained below, Spezyme Ethyl has a very high level of sequence similarity to these parent *Bacillus stearothermophilus* alpha-amylase sequences.

70. As I have also explained above, the '031 patent uses the term "percent homology" interchangeably with "percent identity". I therefore conclude, from the foregoing, that the Spezyme Ethyl alpha-amylase "has at least 95% homology to the parent *Bacillus stearothermophilus* alpha-amylase". In particular, Spezyme Ethyl is at least "95% homologous" (as that term is used in the '031 patent) to the parent *Bacillus stearothermophilus* alpha-amylase from ATCC 31, 195.

C. ***The Spezyme Ethyl Alpha-Amylase Has More Than 95% "Homology" to SEQ ID NO:3***

71. I have been given two additional documents, entitled "GAP ALIGNMENT: SEQ ID NO:3 to Spezyme Ethyl (Old Matrix)" and "GAP ALIGNMENT: SEQ ID NO:3 to Spezyme Ethyl (New Matrix)". Copies of these documents are attached to this Declaration at Exhibits 7 and 8, respectively. I have reviewed both these documents and find that they show an alignment of the Spezyme Ethyl sequence at Exh. 3 to the amino acid sequence of SEQ ID NO:3 in the '031 patent.

72. I understand, on information and belief, that the sequence alignment at Exh. 7 was made with a current version of the GAP computer program (from version 10 of the GCG package) but using what were the default parameters for version 7.3 of that program -- *i.e.*, the version mentioned in the '031 patent at 4:36-45). I therefore understand that the alignment and the other output generated are identical to what a person would have obtained by using version 7.3 of the GAP program with default parameters to align these two sequences.

73. I understand, on information and belief, that the sequence alignment at Exh. 8 was made with a current version of the GAP computer program (from version 10 of the GCG package) using the default parameters for that version. However, when I compare the two alignments and Exhibits 7 and 8, I find that they are identical.

74. The amino acid sequence alignments at Exhibits 7 and 8 both also indicate the "percent identity" that was determined by the GAP program when it made these alignments. In both cases, the percent identities determined are identical: 98.967%, which is greater than 95%. From the foregoing, therefore, I conclude that the Spezyme Ethyl amino acid sequence at Exh. 3 is "has at least 95% homology to SEQ ID NO:3" of the '031 patent.

D. *The Spezyme Ethyl Alpha-Amylase Has More Than 95% "Homology" to Sequence 3 in Figure 1*

75. For the foregoing analysis, I have relied on the amino acid sequence of SEQ ID NO:3 that is set forth in the '031 patent's Sequence Listing. The '031 patent indicates that the amino acid sequence identified as sequence 3 in Figure 1 is also SEQ ID NO:3. *See* the '031 patent at 29:38-45. I notice that there are, in fact, some differences between these two amino acid sequences. As explained below, however, those differences do not affect my analysis or conclusions in this Declaration.

76. In particular, I have been given two additional documents entitled "GAP ALIGNMENT: Sequence 3 (Figure 1) to Spezyme Ethyl (Old Matrix)" and "GAP ALIGNMENT: Sequence 3 (Figure 1) to Spezyme Ethyl (New Matrix)". Copies of these documents are attached to this Declaration at Exhibits 9 and 10, respectively. I have reviewed both these documents and find that they show alignments of the Spezyme Ethyl sequence at Exh. 3 to the amino acid sequence identified as sequence 3 in Figure 1 of the '031 patent.

77. I understand, on information and belief, that the sequence alignment at Exh. 9 was made with a current version of the GAP computer program (from version 10 of the GCG package) but using what were the default parameters for version 7.3 of that program -- *i.e.*, the version mentioned in the '031 patent at 4:36-45. I therefore understand that the alignment and

other output generated are identical to what a person would have obtained by using version 7.3 of the GAP program with default parameters to align these two sequences.

78. I understand, on information and belief, that the sequence alignment at Exh. 10 was made with a current version of the GAP computer program (from version 10 of the GCG package) using the default parameters for that version. However, when I compare the two alignments and Exhibits 9 and 10, I find that they are identical.

79. The amino acid sequence alignments at Exhibits 9 and 10 both also indicate the "percent identity" that was determined by the GAP program when it made these alignments. In both cases, the percent identities determined are identical: 99.587%, which is greater than 95%. From the foregoing, therefore, I conclude that the Spezyme Ethyl amino acid sequence at Exh. 3 is "has at least 95% homology" to sequence 3 in Figure 1 of the '031 patent.

E. Spezyme Ethyl Contains a Deletion of Amino Acids Aligning with Positions 179 and 180 of SEQ ID NO:3

80. As explained above, the alignments of Spezyme Ethyl to the parent ATCC 31,195 alpha-amylase (Exhs. 5 and 6) show that Spezyme Ethyl contains a deletion of two amino acid residues, R179 and G180, from the parent ATCC 31,195 alpha-amylase. The alignments of Spezyme Ethyl with SEQ ID NO:3 (Exhs. 7 and 8) show that these deletions align with residues 179 and 180 in SEQ ID NO:3. Moreover, the deletions in Spezyme Ethyl also align with residues 179 and 180 of Sequence 3 in the '031 patent's Figure 1. *See* Exhibits 9 and 10.

81. This can be readily seen in the document at Exhibit 11, entitled "Alpha-Amylase Alignments". The top sequence in that alignment, which is labeled "Spezyme Ethyl", shows the Spezyme Ethyl amino acid sequence at Exh. 3. The sequences on the second and third lines of the alignment are labeled "SEQ ID NO:3" and "Fig. 1, Seq. 3", respectively, and show those amino acid sequences from the '031 patent, as aligned to Spezyme Ethyl in Exhibits 7-8 (for

alignments of Spezyme Ethyl to SEQ ID NO:3) and Exhibits 9-10 (for alignments to Sequence 3 in Figure 1). The last sequence in the alignment is labeled "ATCC 31,195", and shows that parent alpha-amylase sequence, aligned to Spezyme Ethyl as in Exhibits 5-6.

82. The deletion of amino acids in Spezyme Ethyl is highlighted in Exhibit 11, so it can be more easily seen. This deletion aligns with residues 179-180 of both SEQ ID NO:3 and Sequence 3 from Figure 1 of the '031 patent. The deletion also aligns with residues 179-180 of the parent ATCC 31,195 alpha-amylase sequence.

83. Hence, I find that the Spezyme Ethyl alpha-amylase contains a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering.

F. Spezyme Ethyl Has Alpha-Amylase Activity

84. I notice that Exh. 2 states that:

> [t]he endo-amylase in SPEZYME® ETHYL enzyme randomly hydrolyzes alpha-1,4-glucosidic bonds to quickly reduce the viscosity of gelatinized starch or grain mash, producing soluble dextrins and saccharides under a variety of conditions.

85. I have already explained, alpha-amylase enzymes are characterized has having a particular catalytic activity. In particular, alpha-amylases catalyze a chemical reaction, known as a hydrolysis, by which alpha-1,4-glucosidic bonds in starch are broken. I therefore conclude that the variant alpha-amylase of Spezyme Ethyl has alpha-amylase activity.

IV. SPEZYME ETHYL SATISFIES EVERY LIMITATION IN '031 PATENT CLAIMS 1 AND 3

86. From all of the foregoing, it is my opinion and belief that Genencor's Spezyme Ethyl product contains a variant *Bacillus stearothermophilus* alpha-amylase satisfying each and every limitation of claims 1 and 3 in the '031 patent. This analysis is set forth in detail, *infra*, for each one of those two claims.

A. ***Spezyme Ethyl Satisifes Every Limitation in '031 Patent Claim 1***

87. Genencor has stated that the "SPEZYME ETHYL enzyme contains a thermostable starch hydrolyzing [alpha]-amylase that is derived from a genetically modified strain of *Geobacillus* [*Bacillus*] *stearothermophilus*". As explained above, the Spezyme Ethyl alpha-amylase sequence at Exhibit 3 can be obtained by changing only a few amino acids in the sequence of an original "parent" alpha-amylase from *Bacillus* (*Geobacillus*) *stearothermophilus* strain ATCC 31,195. *See* ¶¶ 59-67, *supra*. Hence, Spezyme Ethyl contains "[a] variant of a parent *Bacillus* [*Geobacillus*] *stearothermophilus* alpha-amylase".

88. The variant enzyme in Spezyme Ethyl, when aligned with the amino acid sequence of the parent ATCC 31,195 alpha-amylase (*e.g.*, using the GAP computer program) has an amino acid sequence that is 100% identical to the sequence of that parent. *See* ¶¶ 68-70, *supra*. Hence, the variant alpha-amylase in Spezyme Ethyl has at least 95% "homology" (as that term is used in the '031 patent") to the parent *Bacillus* [*Geobacillus*] *stearothermophilus* alpha-amylase."

89. The variant enzyme in Spezyme Ethyl, when aligned to SEQ ID NO:3 of the '031 patent (*e.g.*, using the GAP computer program) contains a deletion that aligns with amino acid residues 179 and 180 of SEQ ID NO:3. Hence, the variant enzyme in Spezyme Ethyl "comprises a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering." *See* ¶ 80-83, *supra*.

90. Exhibit 4 states that the Spezyme Ethyl enzyme hydrolyzes alpha-1,4-glucosidic bonds in starch. Hence, the variant enzyme in Spezyme Ethyl "has alpha-amylase activity." *See* ¶ 84-85, *supra*.

91. For the foregoing reasons, therefore, the Spezyme Ethyl product contains a variant of a parent *Bacillus stearothermophilus* alpha-amylase that satisfies each and every limitation in claim 1 of the '031 patent.

B. ***Spezyme Ethyl Satisifes Every Limitation in '031 Patent Claim 3***

92. As explained above, the "SPEZYME ETHYL enzyme contains a thermostable starch hydrolyzing [alpha]-amylase that is derived from a genetically modified strain of *Geobacillus* [*Bacillus*] *stearothermophilus*". *See* Exh. 4. As explained above, the Spezyme Ethyl alpha-amylase sequence at Exhibit 3 can be obtained by changing only a few amino acids in the sequence of an original "parent" alpha-amylase from *Bacillus* [*Geobacillus*] *stearothermophilus* strain ATCC 31,195. *See* ¶¶ 59-67, *supra*. Hence, Spezyme Ethyl contains "[a] variant alpha-amylase".

93. The variant enzyme in Spezyme Ethyl, when aligned with SEQ ID NO:3 of the '031 patent (*e.g.*, using the GAP computer program) has an amino acid sequence that is more than 95% identical to the sequence of that parent. *See* ¶¶ 71-74, *supra*. Hence, the variant alpha-amylase in Spezyme Ethyl has at least 95% "homology" (as that term is used in the '031 patent") to SEQ ID NO:3."

94. The variant enzyme in Spezyme Ethyl, when aligned to SEQ ID NO:3 of the '031 patent, also contains a deletion that aligns with amino acid residues 179 and 180 of SEQ ID NO:3. Hence, the variant enzyme in Spezyme Ethyl "comprises a deletion of amino acids 179 and 180, using SEQ ID NO:3 for numbering." *See* ¶ 80-83, *supra*.

95. Exhibit 4 states that the Spezyme Ethyl enzyme hydrolyzes alpha-1,4-glucosidic bonds in starch. Hence, the variant enzyme in Spezyme Ethyl "has alpha-amylase activity." *See* ¶ 84-85, *supra*.

96. For the foregoing reasons, therefore, the Spezyme Ethyl product contains a variant alpha-amylase that satisfies each and every limitation in claim 3 of the '031 patent.

V. CONCLUSION

97. I declare under penalty of perjury pursuant to the laws of the United States of America that the foregoing statements are true and correct.

Respectfully submitted,

Dated: June 15, 2005

Frances Hamilton Arnold

Frances Hamilton Arnold, Ph.D.

Attachments:

Exhibit 1: *Curriculum Vitae* of Frances Hamilton Arnold, Ph.D.

Exhibit 2: SPEZYME® ETHYL: High Performance Alpha-Amylase for Fuel Ethanol Processes;

Exhibit 3: Spezyme Ethyl Amino Acid Sequence;

Exhibit 4: ATCC 31,195 Alpha-Amylase Amino Acid Sequence;

Exhibit 5: GAP ALIGNMENT: ATCC 31,195 Alpha-Amylase to Spezyme Ethyl (Old Matrix);

Exhibit 6: GAP ALIGNMENT: ATCC 31,195 Alpha-Amylase to Spezyme Ethyl (New Matrix);

Exhibit 7: GAP AlIGNMENT: SEQ ID NO:3 to Spezyme Ethyl (Old Matrix);

Exhibit 8: GAP ALIGNMENT: SEQ ID NO:3 to Spezyme Ethyl (New Matrix);

Exhibit 9: GAP ALIGNMENT: Sequence 3 (Figure 1) to Spezyme Ethyl (Old Matrix);

Exhibit 10: GAP ALIGNMENT: Sequence 3 (Figure 1) to Spezyme Ethyl (New Matrix); and

Exhibit 11: Alpha-Amylase Alignments.

EXHIBIT 1